

Table 1. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm loxP)

Plasmid combo	μ g DNA	Plaques/dish (293 cells)	(Totals)	Plaques/dish (293Cre4 cells)	(Totals)
pCA36:pBHG10	5:5	0, 0, 0, 0		0, 1, 2, 0	
	5:10	0, 0, 0, 1		1, 0, 0, 0	
	10:10	2, 0, 1, 1		1, 2, 0, 0	
			(5)		(7)
pCA36:pBHGloxΔE1,3	5:5	0, 0, 0, 1		0, 0, 0, 0	
	5:10	0, 0, 0, 1		0, 0, 0, 0	
	10:10	0, 0, 2, 1		0, 0, 0, 0	
			(5)		(0)
pCA36lox:pBHG10	5:5	1, 3, 1, 0		0, 1, 0, 1	
	5:10	0, 1, 0, 0		0, 0, 1, 2	
	10:10	0, 0, 0, 0		0, 1, 1, 0	
			(6)		(7)
pCA36lox:pBHGloxΔE1,3	5:5	1, 0, 0, 1		15, 14, 20, 20	
	5:10	0, 0, 0, 0		11, 15, 12, 16	
	10:10	0, 0, 1, 1		18, 9, 10, 8	
			(4)		(168)

Table 2. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ Vectors (\pm loxP)

Plasmid combo	μ g DNA	Plaques/dish (293 cells) (Totals)	Plaques/dish (293Cre4 cells) (Totals)
pCA36:pBHGlox Δ E1,3	5:5	1, 1, 2, 6, 2, 3 (15)	1, 1, 2, 1, 2, 3 (10)
pCA36lox:pBHGlox Δ E1,3	5:5	1, 2, 2, 2, 2, 1 (10)	41,44,41,41,44,31 (242)
pCA36lox Δ :pBHGlox Δ E1,3	5:5	0, 0, 0, 0, 0, 0 (0)	41,36,55,34,24,40 (230)
FG140	1	72, 72	150, 115

Table 3 Efficiency of Ad vector rescue by cotransfection with pBHGloxΔE1,3 and various shuttle plasmids^a

Cell line	Shuttle plasmid	Plaques/dish	Average/dish
293	pCA36lox	6, 2, 3, 3, 5	3.8
	pCA36loxΔ	1, 4, 0, 0, 0	1.0
	pCA36loxΔCreR	2, 2, 4, 3, 2	2.6
	pCA36loxΔCreT	9, 4, 4, 7, 3	5.4
293Cre4	pCA36loxΔ	23, 28, 22, 28	25.3

^a 5μg of all plasmids were used in cotransfections.

Table 4. Efficiency of Ad vector rescue by cotransfection of 293 cells with pBHGloxΔE1,3 and shuttle plasmids encoding Cre^a.

Cell line	Shuttle plasmid	Plaques/dish	Average/dish
293	pCA36lox	2, 3, 1, 0, 1	1.4
	pCA36loxΔ	1, 0, 0, 0, 0	0.2
	pCA36loxΔCreT ^b	3, 1, 5, 2, 4	3.0
	pCA35loxΔCreITR ^b	21, 20, 42, 34, 40	31.4

^aAll cotransfections performed with 5 μg of the indicated shuttle plasmid and 5 μg of pBHGloxΔE1,3

^bPlasmids illustrated in figure 8c.

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Table 5. Efficiency of rescue of fibre and E4 genes into Ad by cotransfection with pFG173lox and pFG23lox^a

Plasmids	$\mu\text{g DNA}$	Number of plaques (average/dish)	
		293 cells	293Cre4 cells
pFG173lox ^b : pFG23dX1lox ^c	5:5	0, 0, 0, 0 (0)	33, 27, 39, 26 (31)
	2:2	0, 0, 0, 0 (0)	9, 15, 10, 9 (11)
pFG173 : pFG23dX1	5:5	0, 0, 0, 0 (0)	0, 0, 1 (0.3)
pFG140	1	95	93

^aCotransfections as diagrammed in figure 9

^bDiagrammed in figure 9b

^cDiagrammed in figure 10

Table 6. Recombinant virus rescue following cotransfection of 293 cells with shuttle plasmids with or without a Cre expression cassette

Plasmid	Number of plaques/dish (average/dish)
pCA36	1, 0, 0, 0 (0.3)
pCA36lox	1, 1, 1, 0 (0.8)
pCA36loxΔ	0, 0, 0, 0
pCA36loxΔCreT	2, 1, 2, 2 (1.8)
pFG140	40, 31 (35.5)

293 cells were cotransfected with 5 μg of pBHGloxΔE1,3 and 5 μg of the indicated shuttle plasmid or 1 μg of pFG140

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Table 7. Efficiency of Ad vector rescue by cotransfection of 293 cells with pBHG10 and shuttle plasmids with a single ITR or an ITR junction

Plasmid	μg of DNA/60 mm	# of Plaques	Average
pCA35:pBHG10	2:2	2, 0, 0, 1, 0, 2	0.83
	5:5	2, 2, 2, 1	1.75
pCA35ITR:pBHG10	2:2	19, 11, 14, 12	14
	5:5	23, 23, 14, 17	19.25
pFG140	1	96, 106	101

Table 8. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

Shuttle Plasmid ^a	Plaques/dish (293 cells)	(Average)	Plaques/dish (293Cre4 cells)	(Average)
pCA36lox	0, 0, 0, 0	(0)	13, 15, 3, 13	(11)
pCA35loxITR	8, 13, 21, 19	(15)	111, 131, 100, 130	(113)
pCA36loxΔ	0, 0, 0, 0	(0)	10, 8, 9, 12	(10)
pCA35loxΔITR	0, 0, 0, 0	(0)	91, 127, 141, 118	(119)
pFG140 ^b	75		83	

^a All cotransfections 5 μ g shuttle plasmid + 5 μ g pBHGlox Δ E1,3

^b 1 μ g/dish

Table 9. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

Plasmids	μ g DNA /dish	Number of plaques /dish (average/dish)	
		293 cells	293Cre4 cells
pCA36:pBHGlox Δ E1,3	5:5	ND	0,3 (1.5)
pCA36lox:pBHGlox Δ E1,3	2:2	ND	9,3 (6)
	5:5	2,0,0,0 (0.5)	30,31,30,30 (30.25)
pCA35lox Δ CreITR:pBHGlox Δ E1,3	2:2	ND	71,60,56,79 (66.5)*
	5:5	36	100,96 (98)
pCA35lox Δ ITR:pBHGlox Δ E1,3	2:2	ND	55,64,75,63 (64.25)*
	5:5	0	120,113 (116.5)
pCA35loxITR:pBHGlox Δ E1,3	2:2	ND	53,54,61,66 (58.5)*
	5:5	ND	130,126 (128)
pFG140 (DC)	1	92	178
pFG140 (CE)	1	94	118

* 5 plaques picked from each of these cotransfections and analyzed. All + for β -gal and all had predicted viral DNA structure

Table 10. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

Plasmids*	ugDNA/dish	Number of plaques/dish (average/dish)	
		293 cells (average)	293 Cre4 cells (average)
pCA36	5	1,0,0,0 (0.3)	1,0,0,0 (0.3)
pCA36lox	5	1,1,1,0 (0.8)	10,18,6,7 (10.3)
pCA36lox Δ	5	0,0,0,0	6,4,3,0 (3.25)
pCA36lox Δ CreT	5	2,1,2,2 (1.8)	4,4,2, (3.3)
pCA35lox Δ CreITR	5	14,23,25,23 (21.3)	116,79,83,100 (94.5)
pCA35lox Δ ITR	5	0,0,0,0 (0)	65,62,64,51 (60.5)
pCA35loxITR	5	4,3,4,0 (2.8)	114,101,75,79 (92.25)
pFG140 (DC)	1	40,31 (35.5)	106,92 (99)
pFG140 (CE)	1	21,19 (20)	44,42 (43)

*cotransfections with 5 μ g pBHGlox Δ E1,3 except for pFG140

Table 11. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

Number of plaques/dish (average/dish)

Genomic plasmid ^a	Shuttle plasmid ^a	293 cells	293Cre4 cells
pBHGlox Δ E1,3	pCA36	2,3,1,2 (2)	3,3,3,1 (2.5)
	pCA36lox Δ	0,0,0,0 (0)	9,23,20,19 (17.8)
	pCA35loxITR	26,27,15,12 (20)	91,101,95,86 (93)
	pCA35lox Δ CreITR	56,42,50,74 (55.5)	94,90,96,92 (93)
pBHGlox Δ E1,3Cre	pCA36	1,1,0,0 (0.5)	2,3,2,0 (1.8)
	pCA36lox Δ	6,5,4,3 (4.5)	20,14,28,24 (21.5)
	pCA35loxITR	77,67,78,76 (74.5)	125,120,130,135 (128)
	pCA35lox Δ CreITR	40,46,47,34 (41.8)	83,90,88,89 (87.5)
pBHGlox Δ E1,3CreR	pCA36	0,0 (0)	ND ^b
	pCA36lox Δ	2,0 (1)	ND
	pCA35loxITR	39,29 (34)	ND
	pCA35lox Δ CreITR	7,6 (6.6)	ND
pFG140		61,52 (56.5)	85,87 (86)

^a Cotransfections with 5 μ g each plasmid/dish except 1 μ g/dish for pFG140

^b Not done

Table 12. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (\pm lox, \pm ITR junction, \pm CRE)

Plaques / dish (average / dish)				
Genomic plasmid	Shuttle plasmid	ug DNA /dish	293 cells	293Cre4 cells
pBHGlox Δ E1,3	pCA36	5:5	2,2,1,0 (1.25)	2,1,1,1 (1.3)
	pCA36lox Δ	5:5	2,0,0,0 (0.5)	26,28,25,27 (26.5)
	pCA35loxITR	2:2 5:5	ND ^a 3,9,6,6 (6)	75,90 (82.5) TNTC ^b
	pCA35lox Δ ITR	2:2 5:5	ND 1,2,1,0 (1)	55,64 (59.5) TNTC
	pCA35lox Δ CreITR	2:2 5:5	ND 33,28,35,31 (31.8)	61,64 (62.5) TNTC
pBHGlox Δ E1,3 Cre	pCA36	5:5	2,2,1,0 (1.25)	4,1,1,0 (1.5)
	pCA36lox Δ	5:5	6,4,4,6 (5)	21,25,21,17 (21)
	pCA35loxITR	2:2 5:5	ND 57,49,45,54(51.3)	90,96 (93) TNTC
	pCA35lox Δ ITR	2:2 5:5	ND 39,45,39,46 (42.3)	75,87 (81) TNTC
	pCA35lox Δ CreITR	2:2 5:5	ND 54,64,41,40(49.8)	117,103 (110) TNTC
pFG140		1	114,96 (105)	125,140 (132.5)

^a Not done

^b Too numerous to count

Table 13. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (CRE expressed from plasmids, by 293 cells, or both)

Genomic plasmid	Shuttle plasmid	μ gDNA/dish	Plaques/dish (average/dish)	
			293 cells	293Cre4 cells
pBHGlox Δ E1,3	pCA35loxITR	5:5 2:2	3, 6, 9, 13 (8) 6, 4, 1, 3 (4)	TNTC ^a (≥ 109) 65, 55, 64, 69 (63)
	pCA35lox Δ ITR	5:5 2:2	0, 0 (0) 1, 0 (0.5)	TNTC (≥ 117) 49, 57, 47, 54 (52)
	pCA35lox Δ CreITR	5:5 2:2	18, 21, 43 (27) 18, 12, 21, 24 (19)	TNTC (≥ 111) 74, 61, 50, 49 (59)
pBHGlox Δ E1,3Cre	pCA35loxITR	5:5 2:2	52, 66, 63, 57 (60) 48, 47, 32, 43 (43)	TNTC (≥ 116) 72, 85, 69, 75 (72)
	pCA35lox Δ ITR	5:5 2:2	40, 36, 32, 63 (43) 48, 43, 52, 46 (47)	TNTC (≥ 122) 93, 104, 106, 100 (101)
	pCA35lox Δ CreITR	5:5 2:2	54, 56, 51 (54) 33, 37, 35, 19 (31)	TNTC (≥ 68) 110, 94, 89, 83 (94)
pFG140		1	114	150

^a Too numerous to count